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Inverse mRNA Expression of the Selenocysteine-Containing Proteins GI-GPx and SeP in Colorectal Adenomas Compared With Adjacent Normal Mucosa

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Abstract: *Four selenocysteine-containing proteins (gastrointestinal glutathione peroxidase, plasma glutathione peroxidase, selenoprotein P, and thioredoxin reductase-α) are expressed in the colonic mucosa. Because of their antioxidant functions, a protective role in colon carcinogenesis is discussed. The aim of this study was to elucidate an involvement of gastrointestinal selenoproteins during the adenoma-carcinoma sequence. Matched pairs of biopsies of colorectal adenomas and adjacent normal mucosa from 11 patients were analyzed for mRNA expression, protein expression, or enzyme activity of selenoproteins by Northern blot, Western blot, or enzymatic tests. All adenomas revealed a marked reduction of selenoprotein P and a variable increase of gastrointestinal glutathione peroxidase mRNA compared with adjacent tissue. Thioredoxin reductase-α and plasma glutathione peroxidase mRNA expression were not altered in adenomas. The Northern blot results were confirmed by Western blot analysis or enzyme activity measurement, respectively. We conclude that gastrointestinal glutathione peroxidase and selenoprotein P play a complementary role in the antioxidative cell defense along the adenoma-carcinoma sequence. It remains to be shown whether upregulation of gastrointestinal glutathione peroxidase in adenomas represents a compensatory mechanism to reduce susceptibility for oxidative damage resulting from the loss of selenoprotein P.*

Introduction

Colorectal tumorigenesis is a multistep process based on inherited or acquired molecular defects that are associated with a progressive disorganization of tissue architecture as well as derangement of regulation of normal cell replication and differentiation. Numerous mutagenic events can occur throughout colorectal carcinogenesis. For instance, activating point mutations in oncogenes such as K-*ras* or loss of heterozygosity in tumor suppressor genes such as APC, MCC, DCC, and p53 facilitate colorectal carcinogenesis (1,2). Additionally, mutations of the "mutator genes" hMSH2, hMLH1, hPMS1, and hPMS2 induce general genomic instability as a result of the impairment of the DNA mismatch repair system (3). A major contribution to DNA damage in tumor progression in the colon (4,5), as well as mismatch repair deficiency (6), is caused by oxidative stress due to production of H_2O_2 and other reactive oxygen species (ROS). The number of ROS-induced hits is estimated at 20,000 per cell per day (7). Several antioxidants, such as reduced glutathione (GSH) and thioredoxin (Trx), are suggested to protect cells and the genome against H_2O_2- and ROS-inducible damage. The proteinaceous tripeptide GSH occurs intracellularly in high concentrations of 0.5–12 mmol (8) and contributes to prevent oxidative decay of cellular and subcellular structures (9). Regeneration of GSH occurs enzymatically by glutathione reductase. Trx, a protein disulfide reductase, catalyzes the first unique step in DNA synthesis and is involved in regeneration of reductive enzymes as well as redox regulation of enzymes and transcription factors (10). Oxidized Trx is regenerated by the selenocysteine-containing protein thioredoxin reductase (TrxR) (11).

Reduction of hydroperoxides is catalyzed by the selenocysteine-containing protein family of glutathione peroxidases (GPx), which use GSH as a cofactor. The classical cellular GPx, cGPx (12,13), the plasma GPx, pGPx (14), and the gastrointestinal GPx, GI-GPx (15), detoxify H_2O_2 and organic hydroperoxides. The reduction of phospholipid hydroperoxide, cholesterol hydroperoxide, and linoleic acid hydroperoxide is catalyzed by phospholipid hydroperoxide GPx (16). In addition, selenoprotein P (SeP) is able to reduce phospholipid hydroperoxide, although less efficiently than phospholipid hydroperoxide GPx (17).

Recently, we identified the mRNA of four different selenocysteine-containing proteins in the mucosa of the gastrointestinal tract: pGPx, GI-GPx, TrxR-α, and SeP (18). pGPx is a secreted GPx isoform (14), which may contribute to

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extracellular neutralization of H_2O_2. In contrast, the cytosolic isoform GI-GPx is suggested to play an important role in controlling cellular redox mechanisms (12,13) and resistance against colon cancer development (19).

Although TrxR-α mRNA has been found nearly ubiquitously in the human digestive tract (18), gastrointestinal expression of the recently described isoform TrxR-β (20) is unknown. Besides control of various cellular redox-related processes such as transcription (e.g., activation of nuclear factor- κB and activator protein-1), TrxR is involved in protein-DNA interactions, growth control, and DNA synthesis (21–23). Furthermore, because of elevated TrxR and Trx gene expression in colon cancer tissue and some cell lines, an association to colon carcinogenesis has been discussed (24,25).

The biological function of SeP, the fourth gastrointestinal selenocysteine-containing protein (18), is discussed. There is increasing evidence for an important role in the antioxidant defense, as indicated by the high content of selenocysteine residues (26–28), a protective effect against diquat- or paraquat-induced liver damage (29,30), protection against peroxynitrite-inducible cell injury (31), protection against oxidative damage from gluthathione depletion in selenium-deficient rats (32), and phospholipid hydroperoxide reduction (17). SeP mRNA expression is detectable in colon carcinoma cells (33). Similar to GI-GPx, particularly high SeP mRNA levels have been demonstrated in the human colon and rectum (18).

To determine a potential involvement of the gastrointestinal selenoproteins in colon cancer development, we investigated human biopsy specimens of colorectal adenomas in comparison to surrounding nonadenomatous tissue for the expression of GI-GPx, SeP, pGPx, and TrxR-α at the mRNA, protein, and functional levels.

Methods

Biopsy Sampling

With written informed consent of the patients, matched pairs of forceps biopsies from colorectal adenomas and adjacent normal mucosa were obtained during therapeutic colonoscopy before removal of the polyps ($n = 10$). Furthermore, biopsies from rectal adenomas were obtained from one patient with familial adenomatous polyposis (FAP) during diagnostic sigmoidoscopy before proctocolectomy. Tissue samples from patients with no pathological findings during diagnostic colonoscopies served as controls.

RNA Isolation and Northern Analyses

Total RNA was isolated by guanidine-CsCl centrifugation according to the method of Chirgwin and co-workers (34). Fifteen micrograms of total RNA were separated by electrophoresis on denaturing formaldehyde gels, transferred to nylon membranes, and fixed by ultraviolet cross-linking. The blots were hybridized in a solution containing 5× saline-sodium citrate, 50% formamide, 1× Denhardt's reagent, 1% sodium dodecyl sulfate, 20 mM sodium phosphate, pH 6.3, 100 μg/ml denatured salmon sperm DNA, and radiolabeled cDNA (probe 0.3 MBq/ml) at 65°C overnight. cDNA probes were labeled with deoxy-[α-^{32}P]CTP (110 TBq/mmol; Amersham, Braunschweig, Germany) by use of the random primed DNA labeling kit (Boehringer, Mannheim, Germany). Integrity and equal loading of RNA were monitored by ethidium bromide staining and hybridization with radiolabeled cDNA probes for the constitutively expressed genes glyceraldehyde 3-phosphate dehydrogenase or β-actin. Blots were exposed to Kodak X-o-mat X-ray films for one to five days at –80°C with intensifying screen. Hybridization signal intensity was evaluated by densitometric measurement.

cDNA Probes

The hybridization probe for human SeP was a 927-bp reverse transcriptase-polymerase chain reaction product amplified from HepG2 cDNA with primers specific for the 5'-part of the published cDNA sequence (35). The full-length cDNA of the human GI-GPx (bases 1–971) was obtained from Dr. F. F. Chu (Duarte, CA) (15). The human fetal liver glyceraldehyde 3-phosphate dehydrogenase cDNA was obtained from American Type Culture Collection (no. 57091). The rat β-actin probe consisted of a 1,200-bp insert and 150 bp of the pBluescript vector obtained from Dr. Mazoub (Boston, MA). The human pGPx probe was polymerase chain reaction cloned (corresponding to bases 70–536) using primers for the published pGPx cDNA sequence (36). The hybridization probe for human TrxR was a differential display polymerase chain reaction product of 1.4 kb or the 2.3-kb 5'-product of rapid amplification of cDNA ends (37).

GPx Activity Measurement

Biopsy specimens were homogenized in a glass Teflon potter, resuspended, and sonicated in buffer containing 250 mM sucrose, 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid, and 1 mM EDTA, pH 7.4. GPx activity was assayed by the method of Beutler (38), with tertiary butylhydroperoxide as the substrate. Cytosols of the biopsies (50 μg) were added to the reaction mixture in a final volume of 1 ml [0.1 M tris(hydroxymethyl)aminomethane (Tris), 0.5 mM EDTA (pH 8), 200 μM NADPH, 2 mM glutathione, and 1 U/ml glutathione reductase type IV from baker's yeast; Sigma Chemical, Deisenhofen, Germany]. The reaction was started by the addition of 7 μM tertiary butylhydroperoxide. After an initial incubation period of 1–1.5 minutes, the oxidation of NADPH was measured at 340 nm, within the linear range of the reaction for 2–3 minutes. The activity of GPx was expressed as nanomoles of NADPH oxidized per minute per milligram of protein, as determined by the Bradford Bio-Rad protein assay (39). Unspecific NADPH oxidation was meas-

ured by the complete inhibition of GPx by the addition of 100 mM mercaptosuccinate, the GPx inhibitor, to the incubation mixture before initiation of the reaction. The background values were subtracted from the obtained results.

TrxR Activity Measurement

TrxR activity was measured according to the dithionitrobenzoic acid (DTNB) method as described by Holmgren and Björnstedt (11). Cytosols were prepared as described above. The assay mixture (1 ml) consisted of 100 mM potassium phosphate, pH 7.0, 10 mM EDTA, 2 mg/ml DTNB, and 0.2 mg/ml NADPH. An aliquot of cytosol (100 µl, 1–6 µg protein/µl) was added, and the change in absorption at 412 nm was monitored for two minutes. Unspecific substrate oxidation (1–8% of the results obtained for the cytosols), as monitored in a reference cuvette containing the assay mixture (900 µl + 100 µl of suspension buffer), was subtracted. Activity was defined as micromoles of DTNB reduced to thionitrobenzoic acid (TNB) per minute ($\Delta 412/13.6 \times 2$). Results are expressed as activity per milligram of cellular protein. Each measurement was performed in triplicate.

Production of Polyclonal Antibodies Against SeP

Antigenic domains of SeP were predicted by antigenicity diagrams (40–42). An antigenic peptide of 15 amino acids was chosen from the COOH region of SeP (43). The peptide was synthesized and coupled to the carrier keyhole limpet hemocyanin (Eurogentec, Seraing, Belgium). Rabbits were immunized and boosted with this conjugate. Immunoglobulins (Ig) were isolated from the rabbit sera by sodium sulfate precipitation according to Kekwick (44); then the samples were subjected to centrifugation and dialysis.

Western Blot Analysis

Biopsy samples were diluted with sample buffer [400 mM Tris, 4% lithium dodecyl sulfate (wt/vol), 30% glycerin (vol/vol), 204 mM mercaptoacetic acid, and 0.02% bromphenol blue, pH 6.8]; 25 µg of protein were loaded per lane. The proteins were separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (45,46), transferred to nitrocellulose membranes, and stained with Ponceau S. Membranes were blocked with 3% nonfat milk powder, 3% bovine serum albumin (BSA), 2% horse serum, and 0.1% Tween 20 in phosphate-buffered saline (PBS) for two hours at room temperature. Membranes were incubated with the different Ig preparations (working dilution 1:750) in PBS, 1% nonfat dry milk, 1% BSA, 1% horse serum, and 0.1% Tween 20 overnight at 4°C and washed with a solution containing 10 mM Tris, 150 mM or 1 M NaCl, 0.1% *t*-octylphenoxypolyethoxyethanol (Triton X-100), 2 mM EDTA, 1% nonfat dry milk, 1% BSA, and 1% horse serum. Finally, membranes were incubated with a peroxidase-labeled secondary antibody (anti-rabbit antibody, dilution 1:2,000, dilution 1:5,000 in PBS, 1% nonfat dry milk, 1% BSA, 1% horse serum, and 0.1% Tween 20) for one hour at 4°C and washed as described above (43). Detection was done with the enhanced chemiluminescence system according to the manufacturers' instructions (Amersham Buchler).

Results

Patient Characteristics

Matched pairs of biopsy specimens of adenomatous polyps and normal adjacent mucosa of 10 patients were examined as well as one adenomatous tissue biopsy of a patient with FAP. Patient characteristics and the histological findings of the polyps are shown in Table 1.

Northern Blot Analyses

GI-GPx mRNA expression was elevated in the biopsy specimens of all adenomas. The mean increase in the quantified GI-GPx mRNA steady-state levels was 4.7 ± 4.6 (SE)-fold (range 1.1–17.7) compared with normal adjacent mucosa (Patients 1–10). In contrast, SeP mRNA expression was markedly decreased in all adenomas. The average reduction

Table 1. Patient Characteristics and Histology

Patient No.	Age, yr	Gender[a]	Histology
1	57	M	Tubulovillous adenoma with moderate atypia
2	58	F	Villous adenoma with moderate atypia
3	38	M	Tubulovillous adenoma with moderate atypia
4	57	M	Tubular adenoma with mild atypia
5	66	F	Tubulovillous adenoma with moderate atypia
6	79	M	Tubulovillous adenoma with mild atypia
7	65	F	Tubulovillous adenoma with severe atypia
8	57	F	Tubular adenoma with mild atypia
9	66	F	Villous adenoma with moderate atypia
10	60	F	Tubulovillous adenoma with moderate atypia
11[b]	38	M	Tubulovillous adenoma with mild dysplasia

a: M, male; F, female.
b: A patient with familial adenomatous polyposis. In this case, biopsies of adjacent normal mucosa were not available. Patient underwent proctocolectomy.

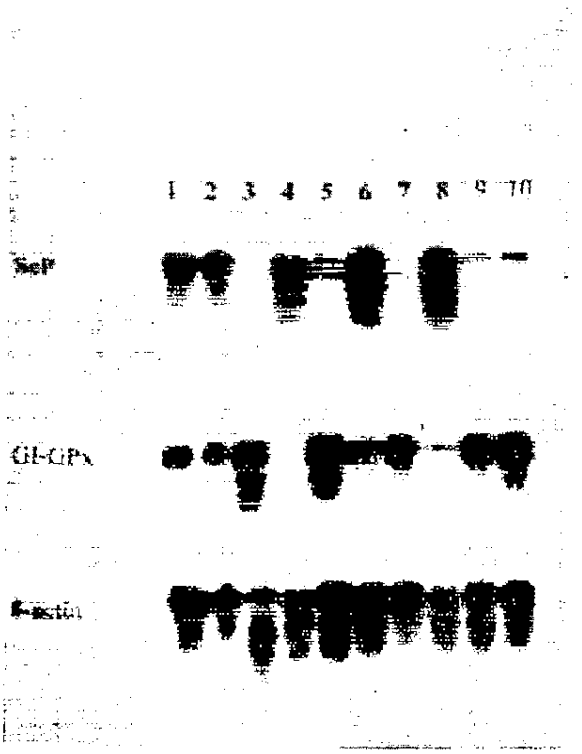

Figure 1. Gastrointestinal glutathione peroxidase (GI-GPx) and selenoprotein P (SeP) mRNA expression in matched pairs of biopsy specimens of colorectal adenomas and adjacent mucosa (representative Northern blot). Northern hybridization of total RNA (15 µg/lane) purified from mucosa biopsies of colorectal adenomas and adjacent tissue are shown. Membrane was hybridized with cDNA probes for mRNA of GI-GPx and SeP. As a reference, blots were hybridized with cDNA for constitutively expressed proteins β-actin and glyceraldehyde 3-phosphate dehydrogenase (not shown). Blots were exposed for 18 h at −80°C. Corresponding RNAs were detected by autoradiography. Lane 1 (control), normal rectal mucosa of a healthy individual; Lanes 2 and 3 (Patient 1), normal mucosa and adjacent tubulovillous adenoma with moderate atypia, respectively; Lanes 4 and 5 (Patient 2), normal mucosa and adjacent villous adenoma with moderate atypia, respectively; Lanes 6 and 7 (Patient 3), normal mucosa and adjacent tubulovillous adenoma with moderate atypia, respectively; Lanes 8 and 9 (Patient 4), normal mucosa and adjacent tubular adenoma with mild dysplasia, respectively; Lane 10 (Patient 11), tubulovillous adenoma with mild dysplasia in a patient with familial adenomatous polyposis.

was a 9.0 ± 9.5-fold decrease (range −3.2 to −32.8) of the SeP mRNA steady-state levels compared with surrounding normal mucosa. The same results were seen in the adenomatous tissue of the patient with FAP (Patient 11; Figures 1 and 2).

In contrast to the marked alterations of GI-GPx and SeP mRNA expression, neither TrxR-α nor pGPx revealed significant differences in the mRNA expression between adenomatous and adjacent nonadenomatous colorectal tissue. Compared with normal surrounding mucosa, TrxR-α and pGPx mRNA levels were only slightly increased in the adenomas: 1.4 ± 0.3-fold (TrxR-α) and 1.5 ± 0.6-fold (pGPx). The deviation of the selenoprotein mRNA levels in the adenomas from the values in the normal mucosa (100%) is shown in Figure 3.

GPx Enzyme Activity

GPx enzyme activity measurements (Patients 1–10) revealed a mean GPx activity in normal adjacent mucosa of 29.8 ± 3.9 nmol NADPH oxidized·min^{-1}·mg protein^{-1}. In adenomatous tissue, a mean GPx activity of 47.6 ± 8.9 nmol NADPH oxidized·min^{-1}·mg protein^{-1} was determined. Therefore, in comparison to the surrounding nonadenomatous mucosa, in colorectal adenomas the enzyme activity levels were increased by a mean factor of 1.6, which is statistically not significant. GPx activities in adenomas and adjacent normal mucosa are shown in Figure 4.

TrxR Activity

In correlation with the Northern blot results, TrxR activity in adenomas was only slightly enhanced but did not differ substantially from that in normal adjacent mucosa. In adenomatous tissue the mean TrxR activity was 2.7 ± 0.4 µmol TNB·min^{-1}·mg protein^{-1}, in contrast to 2.4 ± 0.3 µmol TNB·min^{-1}·mg protein^{-1} in surrounding nonadenomatous tissue (Figure 5).

Ponceau S Staining of Gastrointestinal Proteins and Western Blot Analysis of SeP

The Ponceau S staining showed no differences in the protein pattern or intensity of the signals for extracts of normal mucosa and adenomatous tissue (Figure 6, Lanes 1a and 2a). Variations of signal intensity and pattern of protein bands immunoreactive with SeP antisera were detected by Western blot analysis by Ig preparation of rabbit sera against a COOH regional peptide of SeP (Figure 6, Lanes 1b and 2b). Signals in the normal mucosa extract were stronger than those in the adenoma tissue extract. Several bands were detected with the polyclonal Ig antibody: normal mucosa extracts gave a broad diffuse band between 65 and 51 kDa, and adenoma tissue extracts gave a smaller band between 56 and 51 kDa and a sharp band at 61 kDa. Those molecular mass regions are in accordance with both isoforms of mature human SeP as described in the literature (61 and 55 kDa) (47–50). In both tissue extracts, two sharp bands were detected at 40 and 37 kDa; however, the 37-kDa band was stronger in the normal mucosa tissue extract than in the adenoma tissue extract. A further 34-kDa band was detectable only in the normal mucosa. An additional 24-kDa band was detected in both extracts but was of lower intensity in the adenomatous tissue. These signals in the low-kilodalton range might refer to deglycosylated or immature truncated forms of SeP (31). Furthermore, a band at 81 kDa was visible only in the adenoma tissue extract. The origin of this band is unknown.

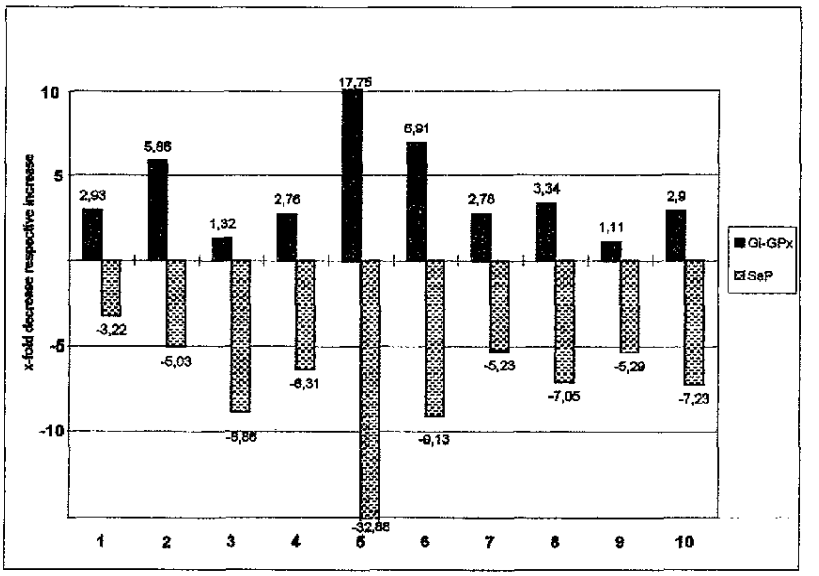

Figure 2. Densitometric evaluation of GI-GPx and SeP mRNA expression in adenomas compared with adjacent normal mucosa. Bars represent intraindividual deviation of GI-GPx and SeP mRNA expression in adenomas compared with adjacent normal mucosa. mRNA steady-state levels were quantified by densitometry.

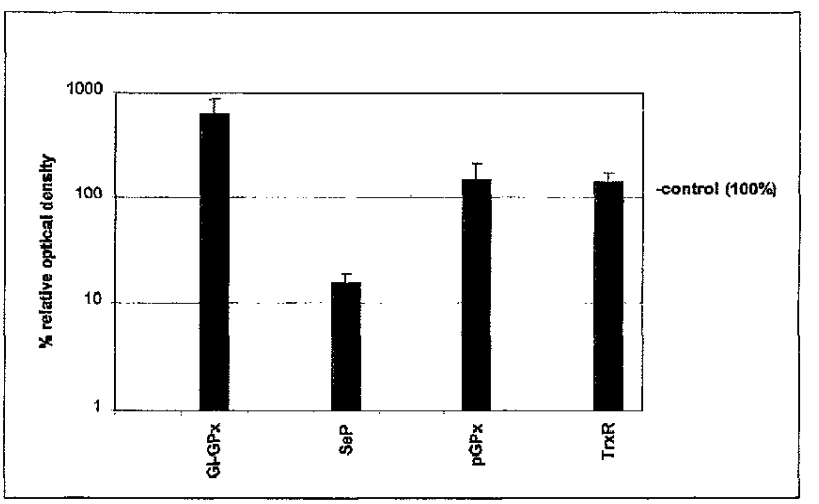

Figure 3. Densitometric evaluation of mean mRNA expression of GI-GPx, SeP, plasma glutathione peroxidase (pGPx), and thioredoxin reductase-α (TrxR-α) (logarithmic scale). Bars represent results (means ± SE) of densitometric analysis of mRNA expression of selenocysteine-containing proteins GI-GPx, SeP, pGPx, and TrxR-α in colorectal adenomas compared with adjacent normal mucosa (100%) ($n = 10$, Patients 1–10). Although mean GI-GPx steady-state level is enhanced to 627 ± 247% and mean SeP steady-state level is decreased to 15.4 ± 3.8%, mean pGPx and TrxR-α mRNA steady-state levels are not substantially altered.

Discussion

Epidemiological data (51,52), animal studies (53–56), and interventional studies (57) have raised an ongoing discussion about a potential chemopreventive effect of selenium against colorectal cancer. We previously described differential mRNA expression of four selenocysteine-containing proteins in the human gastrointestinal tract that may serve as molecular targets for supplemental selenium. Because of a particularly strong expression of GI-GPx and SeP in the colorectal mucosa of healthy individuals, a protective role of those proteins in the development of colorectal cancer is discussed (18). In this study, we present data on the

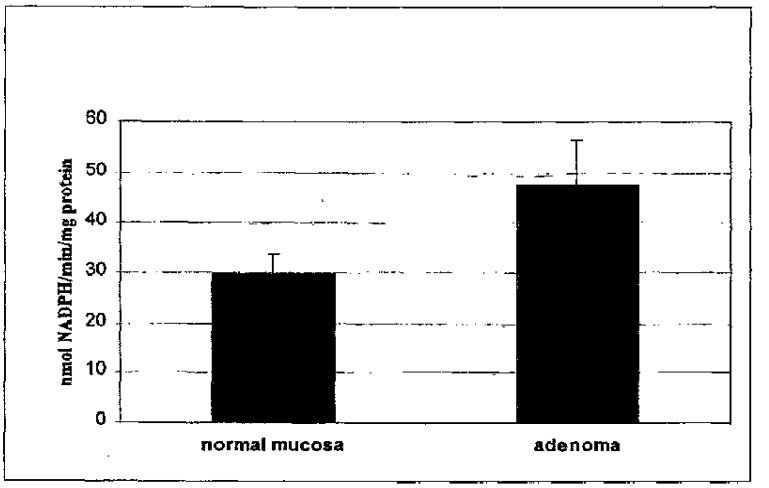

Figure 4. GPx enzyme activity in matched pairs of biopsy specimens of colorectal adenomas and normal mucosa. Activity of GPx in mucosa biopsy specimens of colorectal adenomas and adjacent normal mucosa ($n = 10$) is shown. Values are means ± SE. In colorectal adenomas, mean GPx enzyme activity is moderately increased (1.6-fold, not significant) compared with that in normal adjacent mucosa.

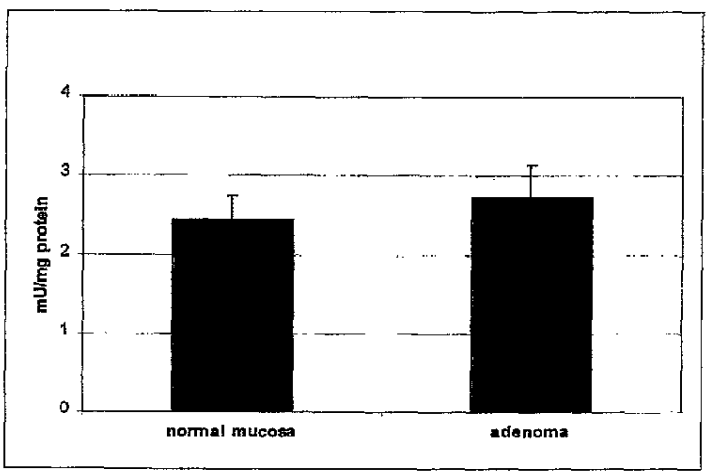

Figure 5. TrxR enzyme activity in matched pairs of biopsy specimens of colorectal adenomas and normal mucosa. Activity of TrxR in mucosa biopsy specimens of colorectal adenomas and adjacent normal mucosa in Figure 4 are shown ($n = 10$). Values are means ± SE; 1 U = 1 μmol thionitrobenzene/min. In adenomas, mean TrxR enzyme activity is only slightly increased (1.1-fold) compared with that in normal mucosa.

mRNA expression, enzyme activity, and protein expression of gastrointestinal selenoproteins in colorectal adenomas compared with normal adjacent mucosa. All adenomatous polyps revealed markedly decreased SeP and elevated GI-GPx mRNA levels. Obviously, the mRNA expression pattern of SeP and GI-GPx changes during adenomatous transition, suggesting links to colon carcinogenesis. Whether their inverse mRNA expression in adenomas is caused by a common regulating factor or represents independent phenomenona must be discussed. The fact that both genes encode for selenocysteine-containing proteins raises the question regarding the role of selenium supply, particularly in reference to data suggesting a correlation between low selenium levels and adenoma and cancer development in the colon (51–59). However, the inverse expression of GI-GPx and SeP and the unchanged mRNA steady-state levels of the other selenoproteins TrxR-α and pGPx indicate that the altered expression of GI-GPx and SeP is independent of individual selenium supply.

With regard to the marked decrease of SeP mRNA in adenomatous tissue, cytokines must be discussed as potential regulatory factors. Cloning and characterization of the human SeP promoter by our group revealed downregulation of SeP expression and promoter activity by the proinflamma-

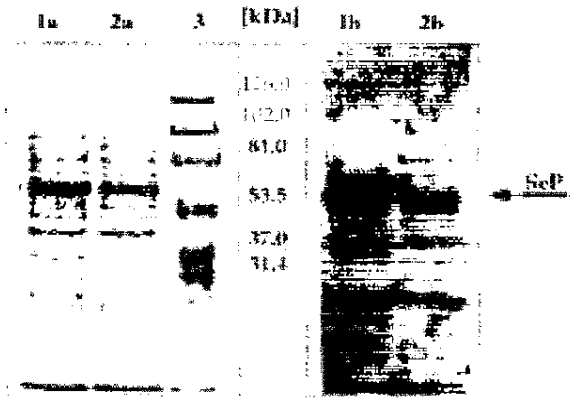

Figure 6. Ponceau S staining for whole protein and Western blot analysis for SeP of tissue extracts from colorectal adenoma and adjacent mucosa. Left: Blotted nitrocellulose membrane stained with Ponceau S: normal colorectal mucosa (Lane 1a) and colorectal adenomatous tissue (Lane 2a). Right: Western blot analysis with immunglobulin preparations from rabbit sera against synthetic COOH regional peptide of SeP: normal colorectal mucosa (Lane 1b) and adenomatous tissue (Lane 2b).

tory cytokines interferon-γ, interleukin-1β, and tumor necrosis factor-α in transient transfection experiments exploiting liver and colon carcinoma cells (60). In addition, we recently presented evidence for a marked inhibitory effect of transforming growth factor-β (TGF-β) on promotor activity of the human SeP gene, mRNA expression, and protein production of SeP (61). Altered production of TGF-β in colon adenomas might be one factor contributing to decreased SeP expression. However, no data support a significant local release of SeP-downregulating cytokines in adenomatous polyps. Stimulatory effects of cytokines or TGF-β on GI-GPx mRNA expression have not been reported. Therefore, other factors, e.g., transcription factors, have to be considered as common regulatory determinants.

Inverse expression patterns of SeP and GI-GPx might represent constitutive epiphenomena associated with adenomatous transition. However, mutagenic abrogation of SeP expression cannot be excluded. The SeP gene was localized to chromosome 5q31 (62), close to the APC gene locus, which is strongly linked to colon carcinoma development. Thus it appears to be intriguing to hypothesize that loss of SeP expression might occur as an early event in the adenoma-carcinoma sequence, consecutively leading to increased susceptibility to oxidative DNA damage and enhanced genetic instability. The GI-GPx gene maps to chromosome 14q24.1 (19,63,64), in the vicinity of the colon cancer susceptibility locus Ccs 1 (65), near Fos and TGFβ3. Data from animal studies indicate a correlation between GI-GPx mRNA levels in the colonic mucosa and resistance against dimethylhydrazine-induced colon cancer (19). According to these data, loss of suppression of GI-GPx expression may represent a mechanism to enhance cancer resistance in an early stage of the adenoma-carcinoma sequence. GI-GPx may contribute to colon cancer resistance by catalyzing the reduction of H_2O_2 and other hydroperoxides in the colonic mucosa (66), which can cause oxidative DNA damage (5,7,67,68). Although there is no doubt about the antioxidant function of GI-GPx, the biological function of SeP is not completely elucidated, but there is increasing evidence for its potential role in antioxidant cell defense (17,26–32). Thus SeP and GI-GPx may act complementarily in the defense against oxidative injury in the colorectal mucosa. Downregulation of SeP does not seem to be useful in conditions of potentially enhanced susceptibility for oxidative damage like adenomatous polyps. However, with the assumption that GI-GPx is the more important antioxidant selenoprotein, downregulation of SeP, which contains up to 10 selenocysteine residues, would possibly spare selenium in favor of GI-GPx. In addition, local intracellular use of selenium by incorporation into GI-GPx might be of greater benefit for the cell than synthesis and secretion of SeP, which would lead to local selenium depletion. According to this hypothesis, abrogation of SeP mRNA and protein expression might be a useful mechanism to optimize defense against ROS-inducible DNA damage in a preneoplastic lesion, which is in jeopardy of forward advancement toward invasive carcinoma with every further hit.

The Western blots confirmed the RNA expression profile for SeP. Western blot analysis revealed a decrease of the SeP signal (51–65 kDa) for adenoma tissue extracts compared with normal mucosa. The different patterns may result from changes in the distribution of several SeP isoforms in adenomatous and nonadenomatous tissue. The detection of more than one signal by polyclonal Ig preparations indicates various stages of processing of SeP. The broad diffuse bands between 65 and 51 kDa for normal mucosa and between 56 and 51 kDa for adenomatous tissue correlate with signals for highly glycosylated mature SeP isoforms (48–50). The sharp 40-, 37-, and 34-kDa signals are likely to represent deglycosylated or immature truncated forms of SeP containing fewer selenocysteine residues (31). Variations in protein pattern and signal intensity in the colorectal mucosa may be related to adenomatous transition and reflect altered function of truncated SeP variants. Formations of truncated or low-molecular-mass proteins have also been described in cell lines infected with human immunodeficiency virus (69).

The increase in GPx enzyme activity was moderate compared with the marked increase in GI-GPx mRNA expression. Because of the constant pGPx mRNA levels and absence of cGPx mRNA in the colonic mucosa (18), the increase of GPx activity is suggested to result mainly from translation of the enhanced transcript GI-GPx levels. Available assays of GPx activity do not discriminate between respective GPx isoforms. In cases with drastically enhanced mRNA expression (e.g., Patient 5), insufficient translation must be discussed potentially due to mRNA instability or perhaps due to a relative intracellular selenium deficiency. Directed by a hairpin loop within the 3'-untranslated region of selenoprotein encoding mRNAs, selenoproteins incorporate selenium as selenocysteine (Sec) by a remarkable translation mechanism, utilizing a unique transfer RNA and

translation factor. The 21st codon UGA, which normally represents an opal stop codon, specifies the incorporation of Sec into protein (70). Therefore, an individual or local selenium deficiency may contribute to an inadequately slight increase in GI-GPx enzyme activity in several individuals as a result of insufficient Sec incorporation during translation. This might be an explanation for several data, which indicate a correlation between low plasma selenium levels and an increased risk of colorectal cancer (51–57). In individual cases, inadequate selenium supply may cause insufficient translation of GI-GPx mRNA, resulting in a lack of GPx. Therefore, low selenium intake may contribute to an increased colorectal carcinoma risk because of an insufficient GI-GPx translation followed by oxidative DNA damage.

In conclusion, changes in mRNA expression patterns of GI-GPx and SeP in colon adenomas suggest their complementary role in the antioxidative defense along the adenoma-carcinoma sequence. The regulating mechanism remains to be shown, but lacking modulations of the other selenoproteins pGPx and TrxR-α indicate that the altered expression of SeP and GI-GPx in adenomas is not directly related to selenium supply. To improve protection against oxidative DNA damage in a susceptible preneoplastic lesion, downregulation of SeP in favor of GI-GPx upregulation may be a useful mechanism. According to this hypothesis, GI-GPx, which is associated with colon cancer resistance, seems to be the more important enzyme for antioxidant defense. Downregulation of SeP, which contains a high amount of selenium, may ensure sufficient local and cellular selenium availability to ensure an increased GI-GPx activity. Loss of SeP mRNA expression as a primary event cannot be excluded. Lack of SeP may cause increased susceptibility to ROS- and phospholipid hydroperoxide-induced DNA damage and, therefore, facilitate tumor progression. The concomitant increase of GI-GPx mRNA levels and enhanced GPx activity in adenomas may represent a compensatory mechanism in the antioxidative cell defense.

Acknowledgments and Notes

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References

1. Vogelstein, B, Fearon, ER, Hamilton, SR, Kern, SE, Preisinger, AC, et al.: Genetic alterations during colorectal tumor development. *N Engl J Med* **319,** 525–532, 1988.
2. Vogelstein, B, and Kinzler, KW: The multistep nature of cancer. *Trends Genet* **9,** 138–141, 1993.
3. Jacoby, RF, Marshall, DJ, Kailas, S, Schlack, S, Harms, B, et al.: Genetic instability associated with adenoma-to-carcinoma progression in hereditary nonpolyposis colon cancer. *Gastroenterology* **109,** 73–82, 1995.
4. Malins, DC, Polissar, NL, and Gunselman, SJ: Progression of human breast cancers to the metastatic state is linked to hydroxyl radical-induced DNA damage. *Proc Natl Acad Sci USA* **93,** 2557–2563, 1996.
5. Cerutti, P: Pro-oxidant states and tumor promotion. *Science* **221,** 375–381, 1985.
6. Jackson, AL, Chen, R, and Loeb, LA: Induction of microsatellite instability by oxidative DNA damage. *Proc Natl Acad Sci USA* **95,** 12468–12473, 1998.
7. Beckman, KB, and Ames, BN: Oxidative decay of DNA. *J Biol Chem* **272,** 19633–19636, 1997.
8. Meister, A, and Anderson, ME: Glutathione. *Annu Rev Biochem* **52,** 711–760, 1983.
9. Fridovich, I: Superoxide radical and superoxide dismutases. *Annu Rev Biochem* **64,** 97–112, 1995.
10. Holmgren, A: Thioredoxin and glutaredoxin systems. *J Biol Chem* **264,** 13963–13966, 1989.
11. Holmgren, N, and Björnstedt, M: Thioredoxin and thioredoxin reductase. *Methods Enzymol* **252,** 199–208, 1995.
12. Flohé, L, Günzler, W, and Schock, HH: Glutathione peroxidase. A selenoenzyme. *FEBS Lett* **32,** 132–134, 1973.
13. Rotruck, JTA, Pope, L, Ganther, HE, Swanson, AB, Hafeman, DG, et al.: Selenium: biological role as a component of glutathione peroxidase. *Science* **179,** 588–590, 1973.
14. Takahashi, K, Avissar, N, Within, J, and Cohen, H: Purification and characterization of human plasma glutathione peroxidase: a selenoglycoprotein distinct from the known cellular enzyme. *Arch Biochem Biophys* **256,** 677–686, 1987.
15. Chu, FF, Doroshow, JH, and Esworthy, RS: Expression, characterization and tissue distribution of a new cellular selenium-dependent glutathione peroxidase, GSHPx-GI. *J Biol Chem* **268,** 2571–2576, 1993.
16. Ursini, F, Maiorano, M, and Gregolin, C: The selenoenzyme phospholipid hydroperoxide glutathione peroxidase. *Biochim Biophys Acta* **839,** 62–70, 1985.
17. Saito, Y, Hayashi, T, Tanaka, A, Watanabe, Y, Suzuki, M, et al.: Selenoprotein P in human plasma as an extracellular phospholipid hydroperoxide glutathione peroxidase. *J Biol Chem* **274,** 2866–2871, 1999.
18. Mörk, H, Lex, B, Scheurlen, M, Dreher, I, Schütze, N, et al.: Expression pattern of gastrointestinal selenoprotein—targets for selenium supplementation. *Nutr Cancer* **32,** 64–70, 1998.
19. Chu, FF, Esworthy, RS, Ho, YS, Bermeister, M, Swiderek,, K, et al.: Expression and chromosomal mapping of mouse GPx2 gene encoding the gastrointestinal form of glutathione peroxidase, GPX-GI. *Biomed Environ Sci* **10,** 156–162, 1997.
20. Spyrou, G, Enmark, E, Miranda-Vizuete, A, and Gustafsson, J: Cloning and expression of a novel mammalian thioredoxin. *J Biol Chem* **272,** 2936–2941, 1997.
21. Arscott, LD, Gromer, S, Schirmer, RH, Becker, K, and Williams, CH, Jr: The mechanism of thioredoxin reductase from human placenta is similar to the mechanism of lipoamide dehydrogenase and glutathione reductase and is distinct from the mechanism of thioredoxin reductase from *Escherichta coli*. *Proc Natl Acad Sci USA* **94,** 3621–3626, 1977.
22. Follmann, H, and Häberlein, I: Thioredoxins: universal, yet specific thiol-disulfide redox cofactors. *Biofactors* **5,** 147–156, 1995.
23. Hirota, K, Matsui, M, Iwata, S, Nishiama, A, Mori, K, et al.: AP-1 transcriptional activity is regulated by a direct association between thioredoxin and ref-1. *Proc Natl Acad Sci USA* **94,** 3633–3638, 1997.
24. Gallegos, A, Berggren, M, Gasdaska, JR, and Powis, G: Mechanisms of the regulation of thioredoxin reductase activity in cancer cells by the chemopreventive agent selenium. *Cancer Res* **57,** 4965–4970, 1997.
25. Berggren, M, Gallegos, A, Gasdaska, JR, Gasdaska, PY, Warneke, J, et al.: Thioredoxin and thioredoxin reductase gene expression in human tumors and cell lines and the effect of serum stimulation and hypoxia. *Anticancer Res* **16,** 3459–3466, 1996.
26. Hill, KE, Lloyd, RS, Yang, JG, Read, R, and Burk, RF: The cDNA for rat selenoprotein P contains 10 TGA codons in the open reading frame. *J Biol Chem* **266,** 10050–10053, 1991.

27. Hill, KE, Lloyd, RS, and Burk, RF: Conserved nucleotide sequences in the open reading frame and 3'-untranslated region of selenoprotein P mRNA. *Proc Natl Acad Sci USA* **90,** 537–541, 1993.
28. Steinert, P, Ahrens, M, Gross, G, and Flohé, L: cDNA and deduced polypeptide sequence of a mouse selenoprotein P. *Biofactors* **6,** 311–319, 1997.
29. Burk, RF, Lawrence, RA, and Lane, JM: Liver necrosis and lipid peroxidation in the rat as the result of paraquat and diquat administration. *J Clin Invest* **65,** 1024–1031, 1980.
30. Burk, RF, Hill, KE, Awad, JA, Morrow, JD, Kato, T, et al.: Pathogenesis of diquat-induced liver necrosis in selenium-deficient rats: assessment of the roles of lipid peroxidation and selenoprotein P. *Hepatology* **21,** 561–569, 1995.
31. Arteel, GE, Mostert, V, Oubrahim, H, Briviba, K, Abel, J, et al.: Protection by selenoprotein P in human plasma against peroxynitrite-mediated oxidation and nitration. *Biol Chem* **379,** 1201–1205, 1998.
32. Burk, RF, Hill, KE, Awad, JA, Morrow, JD, and Lyons, PR: Liver and kidney necrosis in selenium-deficient rats depleted of glutathione. *Lab Invest* **72,** 723–730, 1995.
33. Dreher, I, Schmutzler, C, Jakob, F, and Köhrle, J: Expression of selenoproteins in various rat and human tissues and cell lines. *J Trace Elem Med Biol* **11,** 83–91, 1997.
34. Chirgwin, JM, Przybyla, AE, MacDonald, RJ, and Rutter, WJ: Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18,** 5294–5299, 1979.
35. Hill, KE, Lloyd, RS, Yang, JG, Read, R, and Burk, RF: The cDNA for rat selenoprotein P contains 10 TGA codons in the open reading frame. *J Biol Chem* **266,** 10050–10053, 1991.
36. Yoshimura, S, Suemizu, H, Taniguchi, Y, Arimori, K, Kawabe, N, et al.: The human plasma glutathione peroxidase-encoding gene: organization, sequence, and localization to chromosome 5q32. *Gene* **145,** 293–297, 1994.
37. Schütze, N, Bachthaler, M, Lechner, A, Köhrle, J, and Jakob, F: Identification by differential display PCR of the selenoprotein thioredoxin reductase as a $1\alpha,25(OH)_2$-vitamin D_3 responsive gene in human osteoblasts—regulation by selenite. *Biofactors* **7,** 299–310, 1998.
38. Beutler, E: Glutathione peroxidase (GSH-Px). In *Red Cell Metabolism: A Manual of Biochemical Methods.* New York: Grune & Stratton, 1975, pp 71–73.
39. Bradford, MM: A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **7,** 248–254, 1976.
40. Chou, PY, and Fasman, GD: Prediction of protein conformation. *Biochemistry* **13,** 222–245, 1974.
41. Hopp, TP, and Woods, KR: Prediction of protein antigenic determinants from amino acid sequences. *Proc Natl Acad Sci USA* **78,** 3824–3828, 1981.
42. Hopp, TP, and Woods, KR: A computer program for predicting protein antigenic determinants. *Mol Immunol* **20,** 483–489, 1983.
43. Hesse, K, Dreher, I, Baur, A, Lex, B, Schmutzler, C, et al.: Expression of selenoproteins in various eukaryotic cell lines. In *Spurenelemente*, D Meissner (ed). Stuttgart: Wissenschaftliche Verlagsgesellschaft, 1999, pp 46–48.
44. Kekwick, RA: Observations at the electrophoretic moving boundary with Lamm's scale method. *Trans Faraday Soc* **36,** 47–52, 1940.
45. Lämmli, UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227,** 680–685, 1970.
46. Köhrle, J, Rasmussen, UB, Ekenbarger, DM, Alex, S, Rokos, H, et al.: Affinity labeling of rat liver and kidney type I 5'-deiodase. Identification of the 27-kDa substrate binding subunit. *J Biol Chem* **265,** 6155–6163, 1990.
47. Akesson, B, Bellew, T, and Burk, RF: Purification of selenoprotein P from human plasma. *Biochim Biophys Acta* **1204,** 243–249, 1994.
48. Burk, RF, and Hill, KE: Selenoprotein P. A selenium-rich extracellular glycoprotein. *J Nutr* **124,** 1891–1897, 1994.
49. Chittum, HS, Himeno, S, Hill, KE, and Burk, RF: Multiple forms of selenoprotein P in rat plasma. *Arch Biochem Biophys* **325,** 124–128, 1996.
50. Himeno, S, Chittum, HS, and Burk, RF: Isoforms of selenoprotein P in rat plasma. Evidence for a full-length form and another form that terminates at the second UGA in the open reading frame. *J Biol Chem* **271,** 15769–15775, 1996.
51. Schrauzer, GN, White, DA, and Schneider, CJ: Cancer mortality correlation studies. III. Statistical associations with dietary selenium intakes. *Bioinorg Chem* **7,** 23–34, 1977.
52. Jansson, B: Geographic mappings of colorectal cancer rates: a retrospect of studies, 1974–1984. *Cancer Prev Detect* **8,** 341–348, 1985.
53. Nelson, RL, Abcarian, H, Nelson, TM, Misumi, A, Kako, H, et al.: The effect of dietary selenium deficiency on acute colorectal mucosal nucleotoxicity induced by several carcinogens in the rodent. *Am J Surg* **172,** 185–188, 1996.
54. Jao, SW, Shen, KL, Lee, W, and Ho, YS: Effect of selenium on 1,2-dimethyhydrazine-induced intestinal cancer in rats. *Dis Colon Rectum* **39,** 628–631, 1996.
55. El-Bayoumy, K, Upadhyaya, P, Chae, YH, Sohn, OS, Rao, CV, et al.: Chemoprevention of cancer by organoselenium compounds. *J Cell Biochem Suppl* **22,** 62–100, 1995.
56. Reddy, BS, Upadhyaya, P, Simi, B, and Rao, CV: Evaluation of organoselenium compounds for potential chemoprevention properties in colon carcinogenesis. *Anticancer Res* **14,** 2509–2514, 1994.
57. Clark, LC, Combs, GF, Jr, Turnbull, BW, et al. (for the Nutritional Prevention of Cancer Study Group): Effects of selenium supplementation for cancer prevention in patients with carcinoma in the skin. *JAMA* **276,** 1957–1963, 1996.
58. Russo, MW, Murray, SC, Wurzelmann, JI, Woosley, JT, and Sandler, RS: Plasma selenium levels and the risk of colorectal adenomas. *Nutr Cancer* **28,** 125–129, 1997.
59. Clark, LC, Hixson, LJ, Combs, GF, Jr, Reid, ME, Turnbull, BW, et al.: Plasma selenium concentration predicts the prevalence of colorectal adenomatous polyps. *Cancer Epidemiol Biomarkers Prev* **2,** 41–46, 1993.
60. Dreher, I, Jakobs, TC, and Köhrle, J: Cloning and characterization of the human selenoprotein P promoter. *J Biol Chem* **272,** 29364–29371, 1997.
61. Mostert, V, Dreher, I, Köhrle, J, and Abel, J: Transforming growth factor-β_1 inhibits expression of selenoprotein P in cultured human liver cells. *FEBS Lett* **460,** 23–26, 1999.
62. Hill, KE, Dasouki, M, Phillips, JA, and Burk, RF: Human selenoprotein P gene maps to 5q31. *Genomics* **36,** 550–551, 1996.
63. Chu, FF, Esworthy, RS, and Burmeister, M: The mouse glutathione peroxidase GPX2 gene maps to chromosone 12; its pseudogene GPX2-ps maps to chromosome 7. *Genomics* **33,** 516–518, 1996.
64. Chu, FF, de Silva, HAR, Esworthy, RS, Roteva, KK, Walters, CE, et al.: Polymorphism and chromosomal localization of the GI-form of the human glutathione peroxidase GPX2 on 14q24.1 by *in situ* hybridization. *Genomics* **32,** 272–276, 1996.
65. Jacoby, RF, Hohman, C, Marshall, DJ, Frich, TJ, Schlack, S, et al.: Genetic analysis of colon cancer susceptibility in mice. *Genomics* **22,** 381–387, 1994.
66. Esworthy, RS, Swiderek, KM, Ho, YS, and Chu, FF: Selenium-dependent glutathione peroxidase-GI is a major glutathione peroxidase activity in the mucosal epithelium of rodent intestine. *Biochim Biophys Acta* **1381,** 213–226, 1998.
67. Feig, DI, Reid, TM, and Loeb, LA: Reactive oxygen species in tumorigenesis. *Cancer Res* **54,** 1890s–1894s, 1994.
68. Ames, BN, Shigenaga, MK, and Hagen, TM: Oxidants, antioxidants, and the degenerative diseases of aging. *Proc Natl Acad Sci USA* **90,** 7915–7922, 1993.
69. Gladyshev, VN, Stadtman, TC, Hatfield, DL, and Jeang, KT: Levels of major selenoproteins in T cells decrease during HIV infection and low-molecular-mass selenium compounds increase. *Proc Natl Acad Sci USA* **96,** 835–839, 1999.
70. Low, SC, and Berry, MJ: Knowing when not to stop: selenocysteine incorporation in eucaryotes. *Trends Biol Sci* **21,** 203–208, 1996.